

Figure 1: Alignment of INSP105 against placental growth hormone (*Homo sapiens*).

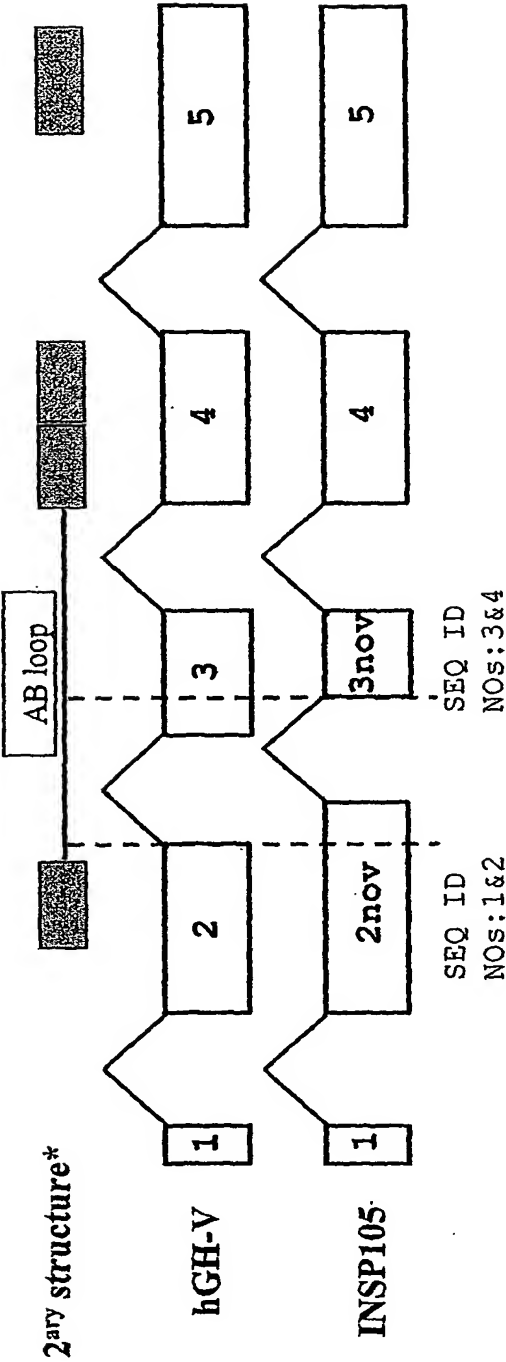
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Query: 1  MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRRRLYQLAYDTYQEFVSS 60
          MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRRRLYQLAYDTYQEF  +
Sbjct: 1  MAAGSRTSLLLAFGLLCLSWLQEGSAFPTIPLSRLFDNAMLRRRLYQLAYDTYQEFEEA 60
                                     ***

Query: 61  WVM-----ESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR 102
          +++                      ESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR
Sbjct: 61  YILKEQKYSFLQNPQTSLCFSESIPTPSNRVKTQQKSNLELLRISLLLIQSWLEPVQLLR 120
          *****

Query: 103 SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLDGSPTGQIFNQSYSKFDTKSHNDD 162
          SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLDGSPTGQIFNQSYSKFDTKSHNDD
Sbjct: 121 SVFANSLVYGASDSNVYRHLKDLEEGIQTLMWRLDGSPTGQIFNQSYSKFDTKSHNDD 180

Query: 163 ALLKNYGLLYCFRKMDKVVETFLRIVQCRSVEGSCGF 199
          ALLKNYGLLYCFRKMDKVVETFLRIVQCRSVEGSCGF
Sbjct: 181 ALLKNYGLLYCFRKMDKVVETFLRIVQCRSVEGSCGF 217
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Figure 2: Gene Structure



*Secondary structure is based on hGH-N structure

Figure 3: Predicted nucleotide sequence of INSP105 with translation

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1  atggctgcag gctcccggac gtcctgctc ctggcttttg gcctgctctg
   m a a g s r t s l l l a f g l l

51  cctgtcctgg cttcaagagg gcagtgcctt cccaaccatt cccttatcca
   c l s w l q e g s a f p t i p l s

101 ggctttttga caacgctatg ctccgcgccc gtcgcctgta ccagctggca
   r l f d n a m l r a r r l y q l a

151 tatgacacct atcaggagtt tgtaagctct tgggtaatgg agtctattcc
   y d t y q e f v s s w v m e s i

201 aacaccttcc aacagggtga aaacgcagca gaaatctaac ctagagctgc
   p t p s n r v k t q q k s n l e l

251 tccgcatctc cctgctgctc atccagtcac ggctggagcc cgtgcagctc
   l r i s l l l i q s w l e p v q l

301 ctacaggagcg tcttcgcca cagcctggtg tatggcgctt cggacagcaa
   l r s v f a n s l v y g a s d s

351 cgtctatcgc cacctgaagg acctagagga aggcattcaa acgctgatgt
   n v y r h l k d l e e g i q t l m

401 ggaggctgga agatggcagc ccccggaactg ggcagatctt caatcagttc
   w r l e d g s p r t g q i f n q s

451 tacagcaagt ttgacacaaa atcgcacaaac gatgacgcac tgctcaagaa
   y s k f d t k s h n d d a l l k

501 ctacgggctg ctctactgct tcaggaagga catggacaag gtcgagacat
   n y g l l y c f r k d m d k v e t

551 tcctgcgcat cgtgcagtgc cgctctgtgg agggcagctg tggttc
   f l r i v q c r s v e g s c g f

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Bold = signal sequence

Figure 4: INSP105 coding exon organization in genomic DNA and position of PCR primers

genomic_seq	4708	CACCTAGCGGCAATGGCTGCAG	INSP105-exon2F	501
virtual_cdna	51	CACCTAGCGGCAATGGCTGCAG		85
genomic_seq	5015	CCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTGGCTTCAAGAGGGCA		5064
virtual_cdna	86	CCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTGGCTTCAAGAGGGCA		135
genomic_seq	5065	GTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTGGACAACGCTATGCTC		5114
virtual_cdna	136	GTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTGGACAACGCTATGCTC		185
genomic_seq	5115	CGCGCCCGTCGCCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGT		5164
virtual_cdna	186	CGCGCCCGTCGCCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGT		235
genomic_seq	5165	AAGCTCTTGGGTAATGGgtgcg....ctcagAGTCTATTCCAACACCTT	INSP105-exon3F	5463
virtual_cdna	236	AAGCTCTTGGGTAATGG.....AGTCTATTCCAACACCTT		270
genomic_seq	5464	CCAACAGGGTGAAAACGCAGCAGAAATCTgtgag....tgcagAACCTAG	INSP105-exon2R	5591
virtual_cdna	271	CCAACAGGGTGAAAACGCAGCAGAAATCT.....AACCTAG	INSP105-exon4F	306
genomic_seq	5592	AGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTG	INSP105-exon3R	5641
virtual_cdna	307	AGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTG		356
genomic_seq	5642	CAGCTCCTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGA		5691
virtual_cdna	357	CAGCTCCTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGA		406
genomic_seq	5692	CAGCAACGTCTATCGCCACCTGAAGGACCTAGAGGAAGGCATCCAAACGC		5741
virtual_cdna	407	CAGCAACGTCTATCGCCACCTGAAGGACCTAGAGGAAGGCATCCAAACGC		456
genomic_seq	5742	TGATGTGGgtgag....tgcagAGGCTGGAAGATGGCAGCCCCCGGACT	INSP105-exon5F	6029
virtual_cdna	457	TGATGTGG.....AGGCTGGAAGATGGCAGCCCCCGGACT		491
genomic_seq	6030	GGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAA	INSP105-exon4R	6079
virtual_cdna	492	GGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAA		541
genomic_seq	6080	CGATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGG		6129
virtual_cdna	542	CGATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGG		591
genomic_seq	6130	ACATGGACAAGGTCGAGACATTCTGCGCATCGTGAGTCCCGCTCTGTG		6179
virtual_cdna	592	ACATGGACAAGGTCGAGACATTCTGCGCATCGTGAGTCCCGCTCTGTG		641
genomic_seq	6180	GAGGGCAGCTGTGGCTTCTAGCTGCCCCGGGTGGCATCCCTGTGACCCCTC		6229
virtual_cdna	642	GAGGGCAGCTGTGGCTTCTAGCTGCCCCGGGTGGCATCCCTGTGACCCCTC	INSP105-exon5R	691

Figure 5: Alignment of INSP105 with pENTR clone-miniprep 6 indicating the position of PCR primers used to re-amplify the correct 5' end of the cDNA.

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                                INSP105-exon2F
                                →
INSP105,      1 ATGGCTGCAGGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTCTGG
pENTR-6,      1 CTGGCTGCAGGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTCTGG
                *****

INSP105,      61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGGACAACGCTATG
pENTR-6,      61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGGACAACGCTATG
                *****

INSP105,      121 CTCCGCGCCCGTCGCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAAGCTCT
pENTR-6,      121 CTCCGCGCCCGTCGCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAAGCTCT
                *****

                                INSP105-5' end-R
                                ←
INSP105,      181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
pENTR-6,      181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTCAC
                *****

INSP105,      241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTC
pENTR-6,      241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCGAGTCGTGGCTGGAGCCCGTGCAGTTC
                *****

INSP105,      301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGCTATCGC
pENTR-6,      301 CTCAGGAGTGTCTTCGCCAACAGCCTGGTGTACGGCGCCTCTGACAGCAACGCTATGAC
                *****

INSP105,      361 CACCTGAAGGACCTAGAGGAAGGCATCCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
pENTR-6,      361 CTCCTAAGGACCTAGAGGAAGGCATCCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
                *****

INSP105,      421 CCCC GGACTGGGCAGATCTTCAATCAGTCTACAGCAAGTTTGACACAAAATCGCACAAAC
pENTR-6,      421 CCCC GGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTTGACACAAAATCGCACAAAC
                *****

INSP105,      481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
pENTR-6,      481 CATGACGCACTGCTCAAGAACTACGGGCTGCTCCACTGCTTCAGGAAGGACATGGACAAG
                *****

INSP105,      541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
pENTR-6,      541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
                *****

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Figure 6: Alignment of INSP105 with pENTR clone-miniprep 10 indicating the position of PCR primers used to re-amplify the correct central region of the cDNA.

```

INSP105      1 ATGGCTGCAGGCTCCCGGACGTCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTCTGG
pENTR-10     1 ATGGCT-----CCGGACGTCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTCTGG
          *****
          *****

INSP105      61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGTACAACGCTATG
pENTR-10     61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCTTATCCAGGCTTTTGTACAACGCTATG
          *****
          *****

INSP105      121 CTCCGCGCCCGTCGCTGTACCAGCTGGCATATGACACCTATCAGGAGTTTGTAAAGCTCT
pENTR-10     121 CTCCGCGCCCATCGTCTGCACCGCTGGCCTTTGACACCTACCAGGAGTTTGTAAAGCTCT
          *****
          *****
                    INSP105-center-F----->
INSP105      181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAACCGCAGCAGAAATCTAAC
pENTR-10     181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAACCGCAGCAGAAATCTAAC
          *****
          *****
->
INSP105      241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTCAGCTC
pENTR-10     241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTCAGCTC
          *****
          *****

INSP105      301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGCTCTATCGC
pENTR-10     301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGCTCTATCGC
          *****
          *****
                    INSP105-center-R-----<
INSP105      361 CACCTGAAGGACCTAGAGGAAGGCATCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
pENTR-10     361 CACCTGAAGGACCTAGAGGAAGGCATCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
          *****
          *****
<-
INSP105      421 CCCCAGTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
pENTR-10     421 CACCTGACTGGGCAGACCTCAAGCAGACCTACAGCAAGTTTGACACAAAATCGCACAAC
          * * *
          * * *

INSP105      481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
pENTR-10     481 CATGACGCACTGCTCAAGAACTACGGGCTGCTCCACTGCTTCAGGAAGGACATGGACAAG
          *****
          *****

INSP105      541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
pENTR-10     541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
          *****
          *****

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Figure 7: Alignment of INSP105 with pENTR clone-miniprep 3 indicating the position of PCR primers used to re-amplify the correct 3' end of the cDNA.

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INSP105      1 ATGGCTGCAGGCTCCCGGACGTCCCTGCTCCTGGCTTTTGGCCTGCTCTGCCTGTCTCTGG
pENTR-3      1 ATGGCTGNAGGNTCCCGGACGTCCCTGNTCCTGGCTTTTGGCCTGGTCTGNCTGNCCTGG
*****
INSP105      61 CTTCAAGAGGGCAGTGCCTTCCCAACCATTCCCTTATCCAGGCTTTTGACAACGCTATG
pENTR-3      61 CTTCAAGAGGGCAGTGNCTTCCCAACCATTCCCTTATCCAGGCTTTTGACAACGCTATG
*****
INSP105      121 CTCCGCGCCCGTCGCCTGTACCAAGCTGGCATATGACACCTATCAGGAGTTTGTAAAGCTCT
pENTR-3      121 CTCCGCGCCCGTCGCCTGTACCAAGCTGGCATATGACACCTATCAGGAGTTTGTAAAGCTCT
*****
INSP105      181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
pENTR-3      181 TGGGTAATGGAGTCTATTCCAACACCTTCCAACAGGGTGAAAACGCAGCAGAAATCTAAC
*****
INSP105      241 CTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCCAGTCATGGCTGGAGCCCGTGCAGCTC
pENTR-3      241 CTAGAGCTGCTCCGCATC-----
*****
INSP105      301 CTCAGGAGCGTCTTCGCCAACAGCCTGGTGTATGGCGCCTCGGACAGCAACGTCTATCGC
pENTR-3      301 -----
*****
INSP105      361 CACCTGAAGGACCTAGAGGAAGGCATCAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
pENTR-3      361 -----CAAACGCTGATGTGGAGGCTGGAAGATGGCAGC
*****
INSP105      421 CCCCGGACTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
pENTR-3      421 CCCCGGACTGGGCAGATCTTCAATCAGTCCTACAGCAAGTTTGACACAAAATCGCACAAC
*****
INSP105      481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
pENTR-3      481 GATGACGCACTGCTCAAGAACTACGGGCTGCTCTACTGCTTCAGGAAGGACATGGACAAG
*****
INSP105      541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
pENTR-3      541 GTCGAGACATTCTGCGCATCGTGCAGTGCCGCTCTGTGGAGGGCAGCTGTGGCTTC
*****
INSP105-3' end-F ----->
INSP105-3' end-R -----<

```

Figure 8: Nucleotide sequence and translation of cloned INSP105 ORF.

```

1  acaagtttgt acaaaaaaagc aggcttcgcc accatggctg caggctcccg
                                m a a g s
51  gacgtccctg ctccctggctt ttggcctgct ctgcctgtcc tggtttcaag
    r t s l l l a f g l l c l s w l q
101 agggcagtgc ctccccaacc attcccttat ccaggctttt tgacaacgct
    e g s a f p t i p l s r l f d n a
151 atgctccgcg cccgtcgcct gtaccagctg gcatatgaca cctatcagga
    m l r a r r l y q l a y d t y q
201 gtttgtaagc tcttgggtaa tggagtctat tccaacacct tccaacaggg
    e f v s s w v m e s i p t p s n r
251 tgaaaacgca gcagaaatct aacctagagc tgctccgcat ctccctgctg
    v k t q q k s n l e l l r i s l l
301 ctcattccagt catggctgga gcccgtcgag ctccctcagga gcgtcttcgc
    l i q s w l e p v q l l r s v f
351 caacagcctg gtgtatggcg cctcggacag caacgtctat cgccacctga
    a n s l v y g a s d s n v y r h l
401 aggacctaga ggaaggcatc caaacgctga tgtggaggct ggaagatggc
    k d l e e g i q t l m w r l e d g
451 agcccccgga ctgggcagat cttcaatcag tcctacagca agtttgacac
    s p r t g q i f n q s y s k f d
501 aaaatcgcac aacgatgacg cactgctcaa gaactacggg ctgctctact
    t k s h n d d a l l k n y g l l y
551 gcttcaggaa ggacatggac aaggctcgaga cattcctgcg catcgtgcag
    c f r k d m d k v e t f l r i v q
601 tgccgctctg tggagggcag ctgtggcttc caccatcacc atcaccattg
    c r s v e g s c g f h h h h h h
651 aaaccagct ttcttgtaga aagtgg

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bold = signal sequence

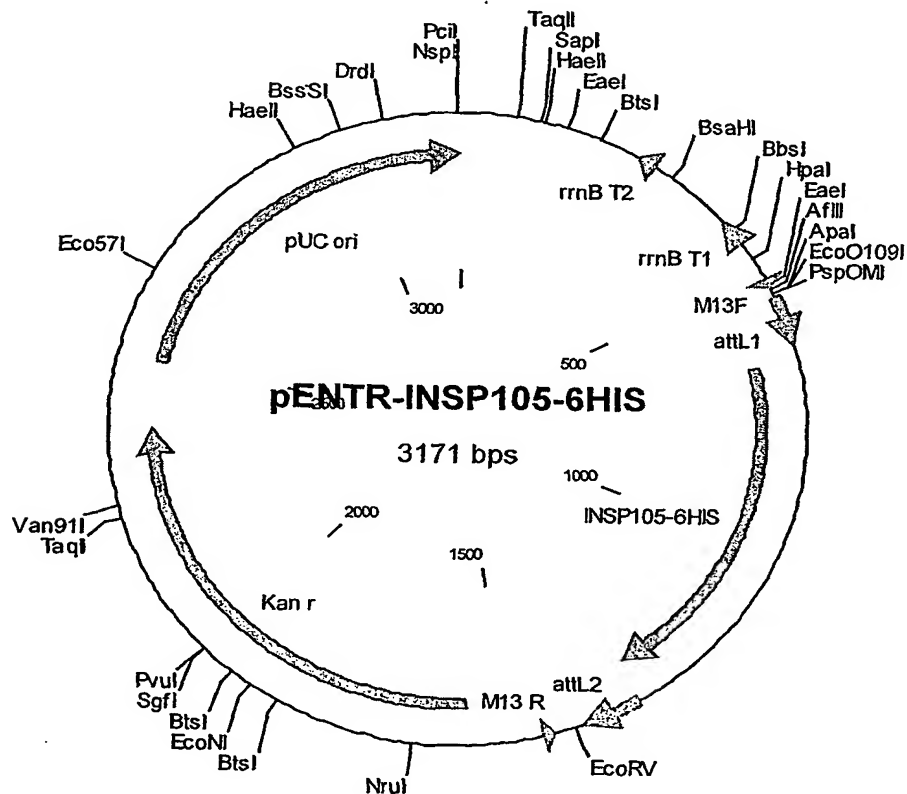
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Figure 9: Map of pENTR-INSP105-6HIS (plasmid 14855)

Molecule: pENTR-INSP105-6HIS, 3171 bps DNA Circular
 File Name: 14855.cm5

Description: Ligation of Blb2-orf.seq* into pDONR221*

Type	Start	End	Name	Description
REGION	295	268	C rrnB T2	transcription termination sequence
REGION	470	427	C rrnB T1	transcription termination sequence
REGION	537	552	M13F	forward primer
REGION	570	651	attL1	
GENE	677	1291	INSP105-6HIS	
REGION	1306	1394	attL2	
REGION	1452	1436	C M13 R	reverse primer
GENE	1565	2374	Kan r	
GENE	2495	3168	pUC ori	



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Figure 10: Map of pEAK12d- INSP105-6HIS (plasmid 14856)

Molecule: pEAK12d-INSP105-6HIS-V1, 7546 bps DNA Circular
 File Name: 14856.cm5

Description: pEAK12 DES with two recombination sites attR1 and attR2 between which the cDNA is inserted

Type	Start	End	Name	Description
REGION	2	595	pmb-ori	
GENE	596	1519	Amp	
REGION	1690	2795	EF-1alpha	
REGION	2703	2722	peak12-F	forward primer
REGION	2855	2874	attB1	
GENE	2888	3484	INSP105-6HIS	
REGION	3492	3513	attB2	
REGION	3520	3948	'A	poly A/splice
REGION	3634	3615	C peak12-R	reverse primer
GENE	4567	3949	C PUR	PUROMYCIN
REGION	4791	4568	C tK	tK promoter
REGION	5286	4792	C Ori P	
GENE	7338	5286	C EBNA-1	
REGION	7339	7538	sv40	

